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TUMORIGENESIS OF MENINGIOMA AND VESTIBULAR SCHWANNOMA AS EVIDENCED BY MOLECULAR GENETICS

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I. INTRODUCTION

A. Meningioma

Meningioma and vestibular schwannoma are both benign intracranial tumors. Meningioma is the most common benign brain tumor and comprises 13–19% of all primary brain tumors[1]. It also comprises 6.5% of the tumors of cerebellopontine (CP) angle where vestibular schwannoma is most frequently seen[2]. Most cases of meningioma are sporadic but familial association has also been documented. Meningioma occurs in up to half of the patients with the predominantly inherited familial syndrome of neurofibromatosis type 2 (NF2)[3]. Multiple meningiomas are also a feature of NF2.

The most frequent sites of occurrence of meningioma include the parasagittal region, the sphenoid ridge and the convex. Another important but less common location is the CP angle where vestibular schwannoma is usually found, as mentioned above.

Meningioma is an extrinsic brain lesion that produces the symptoms from compression of adjacent brain or cranial nerves. True invasion into neural parenchyma is not seen except with malignant transformation that is observed in less than 10% of the cases[4].

B. Vestibular Schwannoma

Schwannoma is a benign neoplasm that originates from Schwann cells. It tends to arise from sensory nerves, and the vestibular portion of the eighth cranial nerve is most commonly involved, giving rise to the vestibular schwannoma that is more commonly referred to as acoustic neuroma.

Vestibular schwannoma exhibits a remarkable biological stability and almost never progresses to malignancy. It accounts for 8% of all intracranial tumors and has an annual incidence of one per 100,000[5]. The majority of the cases (95%) arise as a unilateral sporadic tumor. Familial tumors are frequently multifocal, and bilateral vestibular schwannomas are usually seen in

NF2 patients.

The hallmark clinical presentation of the vestibular schwannoma is unilateral sensorineural hearing loss. As the tumor expands out of the internal auditory canal into the CP angle, it will eventually stretch or compress adjacent nerves and brain stem, producing a variety of symptoms.

C. Neurofibromatosis Type 2 (NF2)

In 1882, von Recklinghausen[6] reported a syndrome which is later referred to as von Recklinghausen's disease. Recent investigation into this disease revealed that the syndrome included two different disease entities-neurofibromatosis type 1 (NF1), with which the original description by von Recklinghausen was consistent, and NF2. The final separation of NF1 and NF2 came in 1987 when the gene for NF1 was localized to chromosome 17[7] and that for NF2 to chromosome 22[8] by linkage analysis.

NF2 is a highly penetrating autosomal dominant tumor syndrome with a birth incidence of 1 in 35,000[9]. NF2 predisposes affected individuals to the development of vestibular schwannoma, schwannoma of other cranial, spinal and peripheral nerves, meningioma, both intracranial and intraspinal, and other central nervous system tumors. The hallmark of NF2 is bilateral and familial vestibular schwannoma, and the majority of the cases present symptoms related to vestibular schwannoma. At least 20% of the cases present complications of cranial meningioma or a spinal tumor.

The NF2 gene was mapped to the long arm of chromosome 22 by linkage analysis, as stated above, and in 1993, two groups independently isolated identical candidate gene[10],[11]. They showed that the NF2 gene comprises 16 exons and encodes a protein of 595 amino acids-designated as merlin or schwannomin. Later, an additional exon which was shown to be alternatively spliced into the transcript has been identified[12]. The exact function of the NF2 protein is as yet undetermined, but it is likely that the NF2 protein is involved in cell-cell or cell-matrix interactions. Therefore, its loss of function could possibly result in a loss of contact inhibition, leading to the transformation into tumor cells.

II. PURPOSE OF STUDY

The purpose of this paper is first to review the basics of molecular genetics in relation to the field of the investigations which the author has conducted using the tumor samples of meningioma and vestibular schwannoma, and then to present the results of these investigations. The results will be discussed and interpreted from the viewpoint of tumorigenesis of both tumors.

III. BASIC KNOWLEDGE OF MOLECULAR GENETICS

A. Human Genome

Each cell in the body contains two sets of genetic instructions of DNA, one inherited from each parent, and each set has some three billion chemical bases. Within each of these two genetic strands, there are 50,000-100,000 genes, and they are separated by sequences of DNA of unknown function. Only around 2% of the total bases provide useful genetic information. This information is spread over 22 pairs of autosomes and two sex chromosomes.

Each gene consists of exons and introns. Exons are the coding sequences of DNA that are copied into the mature mRNA (this process is termed "transcription") and changed into amino acid sequence of a protein (this process is termed "translation"). Thus, the genetic information of the gene is contained in the exons. Within exons, each group of three nucleotides codes for one amino acid, and this is called codon. Each end of an exon is an important site for splicing the strand from the previous exon and to the following exon. This is called splice site. Introns are non-coding sequences of DNA which are not represented in the mature mRNA. Introns thus do not contain genetic information.

B. Genes Involved in Tumor Development

The concept of tumor development as a result of mutations in normal cellular genes that control growth and differentiation is now accepted. These genes are divided into two categories depending on whether they have a positive or negative influence on cell growth. Those with a positive influence are known as proto-oncogene, and those with a negative influence as tumor suppressor gene.

A proto-oncogene becomes an oncogene following a mutation that results in over-production of the gene with a concomitant overproduction of the active growth-promoting protein. This requires a mutation in only one copy of the gene.

Tumor suppressor gene, on the other hand, normally functions to restrain cell growth, and their inactivation promotes uncontrolled cell growth and tumor formation. Normally, inactivation of one copy of the gene does not affect normal cellular growth control, and to promote oncogenesis, inactivation of both copies of the gene is required.

The strongest evidence for the concept of tumor suppressor gene has come from the studies of retinoblastoma by Knudson[13]. He proposed "two hit theory" to explain the development of retinoblastoma, and later this theory was confirmed to apply to the tumor development by the inactivation of tumor suppressor gene. To date, many tumor suppressor genes are reported, including NF2 gene which is responsible for NF2. It is the purpose of this paper to

show that the NF2 gene plays an important role also in the development of both meningioma and vestibular schwannoma.

C. Retinoblastoma Paradigm and Two Hit Theory

Retinoblastoma is a rare childhood malignant tumor of the eye, and it occurs either as multifocal and bilateral tumors with a tendency to run in families or as a unilateral sporadic tumor. The children who suffer from multiple tumors with familial occurrence have inherited an abnormal copy of the retinoblastoma gene from one parent. This mutated gene is therefore present in every cell of the body, but the normal copy inherited from the other parent prevents an abnormal growth of the cell. A single acquired mutation in any retinal cell will however create the potential for uncontrolled growth and promote tumor formation.

In sporadic cases, both copies of the gene are normal at birth and two acquired mutations in a single cell are required to give rise to a tumor. For a tumor to develop in the sporadic cases, "lightening" must strike the same place twice, while with a hereditary tumor with a pre-existing "one hit" only one more hit is required for tumor development. This is Knudson's "two hit theory"[13].

This relationship between familial and sporadic tumors is now well established and it has become apparent that the genes involved in rarer hereditary tumors are also involved in the pathogenesis of commoner non-familial counterpart tumors. Thus, it will be easy to conceive that "two hit theory" will also apply to the development of meningioma and vestibular schwannoma, since both tumors exhibit clinical pictures of commoner form of non-familial unilateral tumor and rarer familial and multiple occurrence, similarly to retinoblastoma cases.

D. Detection of DNA Abnormality

Loss or rearrangement of DNA has been noted in many solid tumors. Gross abnormality can be demonstrated following chromosomal staining and viewing the pathological tissue under light microscope. Smaller deletions of DNA can be detected by looking for the loss of alleles on chromosomes in tumor tissue that are present in constitutional DNA by using particular DNA markers. This is known as loss of heterozygosity (LOH). The markers used here take advantage of natural DNA variations that are usually present in the non-coding sequences of DNA. Mutations within a gene can be confirmed by direct sequencing of the DNA. A new technique called single-stranded conformation polymorphism (SSCP) analysis is proving to be especially useful in screening for unknown mutations.

a) Loss of Heterozygosity (LOH) Study

A variety of mutational mechanisms inactivate a tumor suppressor gene. In many cases, the first mutation is a point mutation or some other small change confined to the gene, while the second mutation is often a large deletion of DNA involving all or part of the chromosome. In such cases, one allele is lost for any marker close to the gene. Thus, if a patient is heterozygous for a marker, the tumor tissue loses heterozygosity while it is retained in constitutional DNA, and this can be detected by LOH study.

DNA samples are amplified by polymerase chain reaction (PCR) with appropriate markers mapping close to the gene. If the target sequences of two DNA strands are of different lengths (heterozygous), PCR amplification produces two discrete bands when submitted to gel electrophoresis. If the tumor tissue has a deletion spaced across the markers in one of the two DNA strands, electrophoresis of paired blood and tumor DNA samples yields two bands in blood DNA but only one band in tumor DNA. This is called "LOH". Detection of LOH by markers mapping close to a particular gene would therefore imply that the gene is acting as a tumor suppressor. If there is no LOH, two bands appear in both blood and tumor DNAs. If the target sequences of two DNA strands are the same (homozygous), electrophoresis of the paired blood and tumor DNAs yields only one band, and we cannot tell whether there is a deletion of DNA sequences in tumor tissue. This is called "uninformative". Examples of LOH, no LOH and uninformative cases are shown in Figure 1.

b) Gene Mutational Study

The most accurate method to detect a mutation in a gene is to perform DNA sequencing for all the bases of the gene. However, this is an extremely laborious and exhausting work, and a screening method is desirable. SSCP analysis is especially useful for screening for a mutation.

A single nucleotide difference between two short single-stranded DNA molecules induces a difference in the conformations adopted by the two strands. This difference is sufficient to produce changes in the molecules' electrophoretic mobility and can be detected as a bandshift on a nondenaturing gel. Thus, by performing SSCP analysis for all of the exons of a gene, mutations within the gene can be screened for. An example of SSCP bandshift will be shown later in Figure 2, when the results of the author's investigations are described.

All DNA samples which show bandshifts by SSCP analysis are then subjected to the determination of base sequences. This is called DNA sequencing. By comparing an abnormal base sequence with the normal one, the site and the extent of a mutation can be determined.

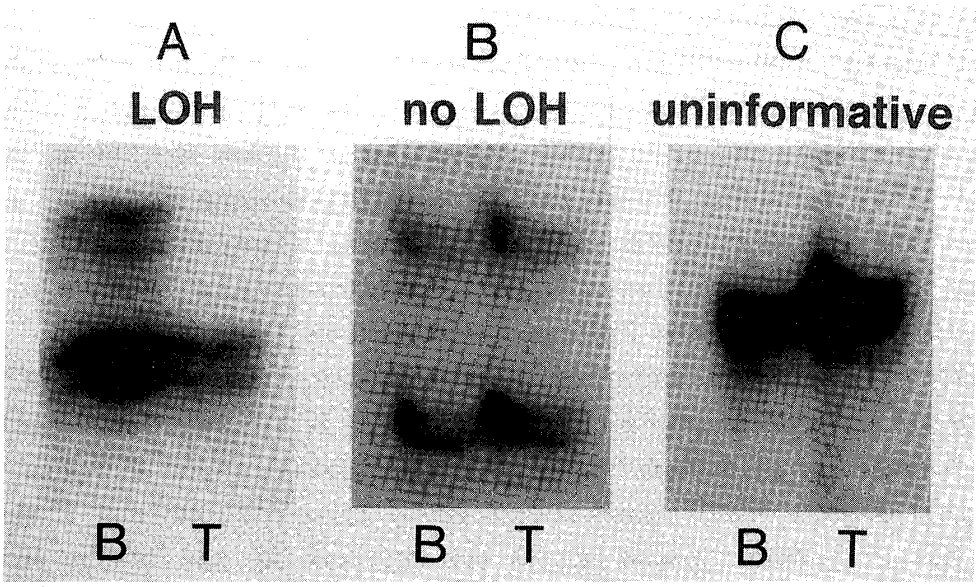

Figure 1 Examples of LOH, no LOH and uninformative cases
A: Presence of two alleles for a particular marker is observed in blood DNA (B) but not in tumor DNA (T). This indicates that there is a loss of one allele due to mutation in tumor DNA. This condition is called loss of heterozygosity (LOH).
B: Retention of heterozygosity is observed in both blood and tumor DNA, indicating no detectable loss of allele in that particular region designated by the marker.
C: When two alleles are identical at a locus, DNA is said to be homozygous for that particular marker, and no information as to allele loss can be obtained.

E. Type of Gene Mutation

A mutation can change the base sequence so that it indicates the termination of protein synthesis. This type of mutation is called a protein truncating mutation. Two kinds of mutations can produce this condition: a frameshift mutation and a nonsense mutation. In the former case, a deletion of a part of the normal sequence of the bases and/or an insertion of some new bases produces a change of reading frame of codons and the resulting succession of new codons becomes different from the normal one. This eventually leads to a stop codon which indicates the termination of protein synthesis. The latter mutation is a substitution of a single base, and this changes a normal codon to a stop codon. Examples of frameshift mutation and nonsense mutation will be shown later in Figures 3 and 4 respectively in the section of the results of the investigations.

When the substitution of a single base leads to a new codon indicating a

change of amino acid, the mutation is called a missense mutation. When the deletion and/or insertion of bases leads to the change of bases in the number of multiples of 3, the reading frame of codons returns to normal after the substituted codons, and the production of protein is not terminated. This is called in-frame mutation. Both types are protein non-truncating mutations, but the protein products themselves are not normal.

Another type of mutation is splice site mutation. In this mutation, the base sequence of the exon itself is normal, but the change of bases at the splice site leads to altered exons or exon skipping. The new splicing thus created often results in a stop codon.

Ⅳ. MOLECULAR GENETIC INVESTIGATIONS OF MENINGIOMA AND VESTIBULAR SCHWANNOMA

A. Background of Study

Extensive cytogenetic studies on meningioma cells undertaken during the last two decades have shown that monosomy of chromosome 22 is the most consistent chromosomal aberration in these cells. One of the conclusions which could be drawn from these studies was that loss of genetic material from chromosome 22 may represent a fundamental event in the tumorigenesis of meningioma.

A number of molecular genetic studies then appeared which confirmed loss of alleles in the tumor material of meningioma. These earlier works demonstrated that the loss or rearrangement of chromosome 22 is a primary event in the tumorigenesis of meningioma, and suggested the existence of a responsible gene acting as a tumor suppressor. The area of this tumor suppressor gene was then located within the region of the NF2 gene[14]. These studies were accelerated by the isolation of the NF2 gene in 1993[10],[11], and more recent molecular genetic investigations have been focused on the detection of NF2 gene mutations in meningioma cells.

On the other hand, the first study which indicated loss of genes on chromosome 22 in sporadic vestibular schwannoma cells was reported in 1986[15], and the finding was then confirmed successively by other LOH studies of a similar kind. These studies suggested that sporadic vestibular schwannoma, as well as schwannoma of NF2 patients, also occurs as a result of mutation of the NF2 gene. Following the isolation of the NF2 gene[10],[11], these LOH studies on chromosome 22 have been replaced by the direct search of mutations of the NF2 gene, and a number of studies have appeared which suggested that the NF2 gene functions as a recessive tumor suppressor gene and that the inactivation of the NF2 gene underlies the tumorigenesis of both inherited and sporadic forms of vestibular schwannoma.

The above review of the previous studies suggests that a mutation of the

NF2 gene is a fundamental step in the tumorigenesis of both meningioma and vestibular schwannoma, not only in familial NF2 but also in sporadic tumor patients. Based on this background, the following molecular genetic investigations on meningioma and vestibular schwannoma were undertaken.

B. Materials and Methods

a) Materials

(i) Meningioma Study

Twenty-three unrelated patients with a diagnosis of sporadic meningioma, 3 patients with hemangioblastoma and one patient with hemangiopericytoma were analyzed in this study. Out of 23 meningiomas, 19 were intracranial tumors and 4 were of spinal cord origin. Hemangioblastoma and hemangiopericytoma were included in this study because of somewhat controversial view that both tumors share a common histogenesis with meningotheliomatous and fibroblastic types of meningioma.

A blood sample was obtained from each patient for constitutional DNA. Tumors excised at surgery were frozen in liquid nitrogen immediately after the surgery, and stored at -70℃. DNA was extracted from both blood and tumor samples by conventional method, and these paired DNA samples constituted the materials of the study.

(ii) Vestibular Schwannoma Study

Ninety-four unrelated patients of schwannoma were analyzed for this study. They included 91 patients with unilateral non-familial vestibular schwannoma, two NF2 patients with bilateral vestibular schwannoma and one patient with vagal neurinoma which is histologically identical to the vestibular schwannoma.

Tumor DNA was extracted from surgical specimens of all patients, while blood DNA was obtained only from 87 patients. Therefore, the comparison of blood and tumor DNA was not possible in 7 patients who all had unilateral non-familial vestibular schwannoma.

b) Methods

(i) LOH Study

Three sets of microsatellite markers (D22S268, D22S280, D22S300) which map close to the NF2 gene on the long arm of chromosome 22 were used for LOH study of meningioma. For vestibular schwannoma study, 8 sets of microsatellites (CYP2D8P, D22S264, D22S268, D22S273, D22S275, D22S280, D22S300, D22S304) were used.

The target section of the DNA flanked by the primers was amplified by PCR using radioactive isotope γ-^{32}P under appropriate conditions, and PCR

products were run on polyacrylamide gel electrophoresis. The gel was then put on an X-ray film for autoradiography to visualize the bands.

(ii) NF2 Gene Mutational Study

All 17 exons of the NF2 gene were separately amplified by PCR using primers and conditions specific to each exon. The PCR products were subsequently analyzed by SSCP and silver staining. PCR products showing aberrant SSCP bands in tumor DNA and normal patterns in constitutional DNA were purified and subjected to sequencing by using *fmol*™ DNA sequencing system with γ-^{32}P labeling. The results were then visualized by autoradiography.

C. Results

a) Meningioma Study

All cases were informative for assessment of chromosome 22 LOH at least at one locus, and LOH was found in 14 cases of meningioma, accounting for 61% of 23 meningioma cases in total. However, no LOH was identified in hemangioblastoma and hemangiopericytoma. In only two cases, LOH was noted for only one marker, and in the rest of the cases with LOH, the other markers either showed LOH or were uninformative, suggesting a larger range of chromosome deletion. These results are tabulated in Table 1.

SSCP analysis of all 17 exons of the NF2 gene revealed bandshifts in 8 of 23 meningiomas (35%), but in none of hemangioblastoma and hemangiopericytoma. The presence of NF2 gene mutation was subsequently confirmed in all 8 tumors by DNA sequencing. In all of the cases, NF2 gene mutation was somatic. It was present only in tumor DNA and not in blood DNA. Seven of 8 mutations were expected to result in truncated protein products (frameshift and nonsense mutations), while, in the remaining one case, abnormal sequence of amino acids was expected (in-frame mutation). The details of the observed mutations are shown in Table 2. All 8 cases of NF2 gene mutation also showed chromosome 22 LOH. This indicates that 55% of cases with chromosome 22 LOH (14 cases) showed concomitant NF2 gene mutations in tumor DNA.

b) Vestibular Schwannoma Study

Chromosome 22 LOH study was conducted for 87 cases where the comparison of blood and tumor DNA was possible. All 87 cases were informative at least at one locus, and LOH was found in 35 cases (40%).

NF2 gene mutational study was conducted in all 94 cases. SSCP analysis of all 17 exons of the NF2 gene revealed bandshifts in 36 tumors (38%), and the presence of 40 NF2 gene mutations were confirmed in these tumors by DNA sequencing. This indicates that two different mutations were observed

Table 1 Results of chromosome 22 LOH study in meningioma group

Case No	Microsatellite Markers		
	D22S268	D22S280	D22S300
MENINGIOMA			
1	LOH	LOH	U
2	LOH	LOH	no
3	U	no	no
4	no	no	no
5	LOH	LOH	LOH
6	no	no	no
7	LOH	U	U
8	no	no	no
9	LOH	LOH	no
10	LOH	LOH	U
11	LOH	U	LOH
12	U	LOH	LOH
13	U	LOH	LOH
14	U	LOH	LOH
15	U	LOH	LOH
16	no	no	no
17	no	no	no
18	U	no	U
19	LOH	LOH	LOH
20	no	U	U
21	LOH	LOH	LOH
22	no	U	no
23	LOH	U	LOH
HEMANGIOBLASTOMA			
24	no	no	no
25	U	no	no
26	no	no	U
HEMANGIOPERICYTOMA			
27	no	no	no

LOH: LOH is noted, no: LOH is not noted, U: uninformative

Table 2 Details of NF2 gene mutations in meningioma study

Case No	Exon (Codon)	Type of Mutation	Chromosome 22 LOH
2	2 (46-48)	in-frame mutation	+
7	11 (341)	nonsense mutation	+
9	1 (9-38)	frameshift mutation	+
10	5 (153)	nonsense mutation	+
11	12 (408-412)	frameshift mutation	+
12	2 (44)	nonsense mutation	+
13	5 (153)	nonsense mutstion	+
21	8 (240-246)	frameshift mutation	+

in 4 tumors. In all of the cases, NF2 gene mutation was somatic and was present only in tumor DNA and not in blood DNA, including two NF2 cases. Thirty-six of 40 mutations were expected to result in truncated protein products (frameshift and nonsense mutations). In the remaining 4 mutations, abnormal sequence of amino acids was expected (one missense mutation, one in-frame mutation, two splice site mutations). The details of these mutations are summarized in Table 3. The distribution of NF2 gene mutations was widely spread over 12 exons, but no mutation was identified in 5 exons including the last three. By looking at the distribution of mutations over codons, a possible existence of some hot spots is suggested (Codons 51-57 and 196-198).

Figure 2 is an example of SSCP bandshift observed in Exon 14 of Case 37 of vestibular schwannoma study. Figure 3 shows a frameshift mutation revealed by the sequencing of this sample. Figure 4 is an example of nonsense mutation (Exon 3 of Case 23).

Not all of the detected mutations were observed in the cases which showed LOH. There were 14 cases in which NF2 gene mutations were observed without LOH and 16 cases of LOH without mutations. Thus, the total number of

Table 3 Details of NF2 gene mutations in vestibular schwannoma study

Exon	No of Mutations	Type of Mutation (Codon No)	
1	2	frameshift (13-20)	frameshift (20-23)
2	6	missense (45)	frameshift (47-58)
		frameshift (51-58)	in-frame (56-61)
		nonsense (57)	nonsense (57)
3	6	splice acceptor	frameshift (89-90)
		frameshift (91)	frameshift (91)
		nonsense (111)	nonsense (121)
4	2	frameshift (143)	nonsense (144)
5	0		
6	5	nonsense (196)	nonsense (196)
		nonsense (196)	frameshift (198)
		splice donor	
7	1	frameshift (205)	
8	5	frameshift (250-255)	frameshift (257-263)
		frameshift (258-259)	frameshift (258-260)
		nonsense (266)	
9	3	frameshift (281)	frameshift (284)
		frameshift (295)	
10	1	frameshift (317)	
11	0		
12	3	frameshift (388-403)	frameshift (419-423)
		frameshift (445-446)	
13	4	frameshift (450)	frameshift (458)
		nonsense (466)	frameshift (473)
14	2	frameshift (489-492)	frameshift (511-514)
15	0		
16	0		
17	0		

the cases in which either chromosome 22 LOH or NF2 gene mutation was identified was 52, accounting for 55% of the total cases. These details are shown in Table 4.

Table 4 Results of NF2 gene mutational study and chromosome 22 LOH study in vestibular schwannoma

Case No	Mutation	LOH	Case No	Mutation	LOH	Case No	Mutation	LOH
1	-	+	34	+	-	67	-	-
2	+	+	35	-	-	68	+	+
3	+	+	36	-	-	69	-	+
4	+	+	37	+	-	70	-	-
5	+	-	38	-	-	71	-	+
6	+	+	39	-	+	72	-	-
7	+	+	40	+	-	73	-	-
8	+	-	41	-	-	74	-	+
9	-	-	42	-	-	75	+	-
10	-	-	43	-	-	76	-	-
11	+	+	44	-	+	77	-	-
12	-	+	45	+	+	78	-	-
13	-	-	46	+	-	79	-	-
14	+	-	47	-	-	80	-	-
15	-	-	48	+	-	81	+	-
16	-	-	49	-	-	82	+	+
17	-	+	50	-	+	83	-	-
18	+	+	51	-	-	84	-	+
19	-	-	52	+	-	85	-	U
20	-	+	53	+	+	86	+	U
21	-	-	54	-	-	87	+	U
22	-	+	55	+	+	88	-	U
23	+	+	56	-	-	89	+	U
24	-	-	57	-	+	90	-	U
25	-	-	58	+	+	91	-	U
26	-	-	59	-	-	92	+	-
27	-	-	60	-	-	93	+	-
28	+	-	61	-	-	94	+	+
29	-	-	62	-	+			
30	+	+	63	+	+			
31	+	+	64	-	-			
32	-	-	65	+	+			
33	-	+	66	-	+			

U: uninformative; Cases 92 & 93 are NF2, and Case 94 is vagal neurinoma

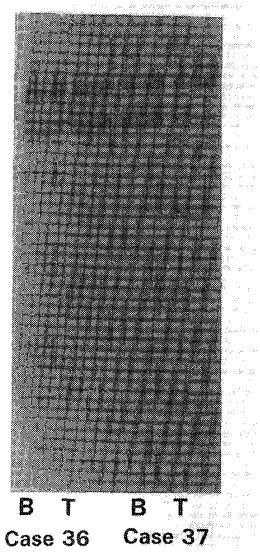

Figure 2 Example of SSCP bandshift
Bandshift is observed only in tumor DNA (T) of Case 37 while blood DNA (B) and Case 36 show normal pattern in Exon 14 analysis of vestibular schwannoma study. This abnoramlity suggests the presence of a mutation in tumor DNA of Case 37.

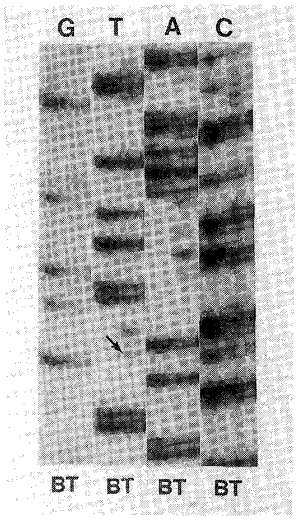

Figure 3 Example of frameshift mutation
Sequencing of the same sample as shown in Figure 2 (Exon 14, Case 37) reveals base deletion in tumor DNA (T) and normal sequence in blood DNA (B). Normal sequence is (from below to above) ...CCAGCACCGTTGCCTCCT GACA... while abnormal sequence in tumor DNA is ...CCAGTCTCCT GACA... This means that CACCGTTGC in normal sequence is replaced by T in abnormal sequence. This replacement results in deletion of 8 bases in total and leads to the shift of reading frames. This is an example of frameshift mutation. Arrow indicates the beginning of abnormality.
G: guanine, T: thymine, A: adenine, C: cytosine

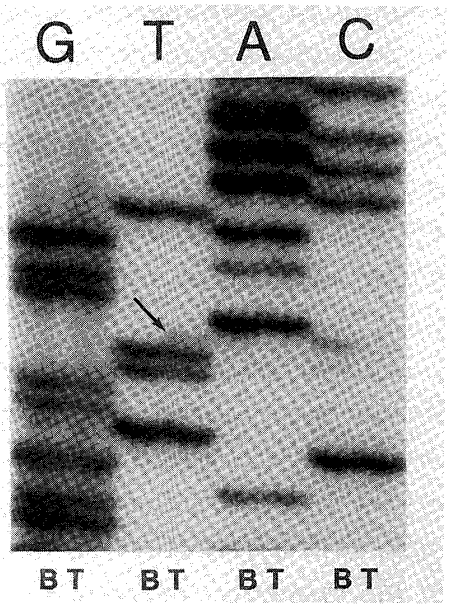

Figure 4 Example of nonsense mutation
Arrow indicates a base change from C to T in tumor DNA (T) resulting in a stop codon. Blood DNA (B) shows normal sequence.
Exon 3, Case 23 of vestibular schwannoma study
G: guanine, T: thymine, A: adenine, C: cytosine

V. DISCUSSION

A. Meningioma Study

The results of the investigations described in the previous section indicated that 61% of meningiomas demonstrated chromosome 22 LOH and 35% showed NF2 gene mutation. These figures are similar to those reported by others[16]–[19]. As was the case with this study, these other studies also reported that all or majority of the cases with NF2 gene mutations showed concomitant chromosome 22 LOH. This would indicate the presence of a mutation in one allele of the paired chromosomes and at least a partial loss of DNA in the other.

Thus, this study confirmed the results of previous studies and showed that chromosome 22 allele loss and somatic NF2 gene mutation are frequent events in sporadic meningioma. These results suggest that the mechanism underlying the tumorigenesis of sporadic meningioma complies with the "two hit" mutation model of Knudson[13], and support the view that the NF2 gene acts as a tumor suppressor gene and that its inactivation is important in the pathogenesis of sporadic meningioma. This view is also supported by a recent report which investigated merlin (schwannomin) expression in sporadic meningioma[20],[21].

The present study, along with other studies, revealed that about 40% of

meningiomas do not demonstrate evidence of chromosome 22 allele loss or NF2 gene mutation. Although not all deletions would necessarily be detected by LOH analysis, this would indicate the possibility that a secondary gene on chromosome 22 or on other chromosomes might be the primary target for mutations in a subset of meningiomas. A recent report implicates chromosomes 1 and 3 as loci of putative tumor suppressor gene[22]. Yet another report suggests the possible presence of alternative mechanism for inactivating merlin (schwannomin) other than gene mutation[23].

Histologically, meningioma is classified into 4 types of benign meningioma, atypical meningioma and malignant meningioma. Recent studies appear to suggest that the inactivation of the NF2 gene would be important in the initiation of tumorigenesis of meningioma and that other genetic events might be involved in determining progression and aggressivity of the tumor. This would corroborate the hypothesis that the formation of aggressive meningioma follows a model of multi-step tumor progression[24].

Hemangioblastoma and hemangiopericytoma were included in this study, and did not show any evidence of chromosome 22 or NF2 gene abnormalities. There was once a controversy that both tumors share a common histogenesis with meningotheliomatous and fibroblastic types of meningioma. Although the number of the cases investigated in this study is very limited, the results support the current view that hemangioblastoma and hemangiopericytoma are tumors not related to meningioma.

B. Vestibular Schwannoma Study

The results of the above-stated investigations revealed that 55% of vestibular schwannomas demonstrated chromosome 22 LOH and/or NF2 gene mutation. This is largely in accordance with other reports of a similar kind of investigations[25]-[27]. These results suggest that vestibular schwannoma shares a common molecular genetic basis of tumorigenesis with meningioma. In both types of tumors, the NF2 gene acts as a tumor suppressor and the mechanism of the tumorigenesis is consistent with the "two hit" mutation model of Knudson[13].

However, the mechanism of tumorigenesis may not be completely the same in these two tumors. While all mutations in meningioma were accompanied by concomitant chromosome 22 LOH, some of the mutations in vestibular schwannoma did not demonstrate chromosome 22 LOH. This situation appears to be largely the same in other reports. Thus, the implications of the vestibular schwannoma cases which did not demonstrate chromosome 22 LOH or NF2 gene mutations might not be the same as in meningioma study. Review of the literature regarding vestibular schwannoma does not yield any recent and important information regarding NF2 gene mutations, and so far, a

possible existence of abnormalities of other loci on chromosome 22 or on other chromosomes is not yet suggested.

One of the big differences between meningioma and vestibular schwannoma is that the latter tumor, unlike meningioma, is histologically uniform and demonstrates remarkable molecular stability. Progression to malignancy is quite rare. Despite this most exclusively benign nature, vestibular schwannoma shows great variations in mode and rate of growth clinically. However, correlation of genetic abnormalities with tumor size and growth index did not disclose any significant relationship[28]. This suggests that the observed heterogeneity is not related to the nature of chromosome 22 abnormality and/or NF2 gene mutations. Thus, a hypothesis would be supported that inactivation of the NF2 gene is likely to be an early event in the tumorigenesis of vestibular schwannoma. Therefore, it seems likely that the NF2 gene is inactivated in the majority of tumors and that this is as early cellular initiating event. Growth and progression, however, appear to be independent of this, and other factors need to be investigated.

One case of vagal neurinoma, which is histologically identical to vestibular schwannoma, was included in this study, and showed both chromosome 22 LOH and NF2 gene mutation. Thus, schwannoma originating from nerves other than the eighth cranial nerve also appears to show abnormalities of chromosome 22 and/or the NF2 gene, as in the case of vestibular schwannoma.

Two NF2 cases included in this study did not demonstrate any germline mutation. So far, even with efficient screening methods, only a proportion of patients have demonstrated germline mutations, and the cases of this study do not contradict the clinical diagnosis of NF2.

Ⅵ. SUMMARY

NF2 is caused by mutations of the NF2 gene. The NF2 gene was mapped to the long arm of chromosome 22 and has recently been isolated. Although meningioma and vestibular schwannoma are usually sporadic tumors, bilateral vestibular schwannoma is a hallmark feature of NF2 and meningioma also occurs frequently in NF2 patients.

In this paper, the basics of some fields of molecular genetics were first reviewed, and then, the results of the investigations which the author has conducted on meningioma and vestibular schwannoma were presented.

For meningioma study, 23 sporadic meningiomas, 3 hemangioblastomas and one hemangiopericytoma were investigated. For vestibular schwannoma study, 91 sporadic vestibular schwannomas, 2 vestibular schwannomas from NF2 patients and one vagal neurinoma (histologically identical to vestibular schwannoma) were investigated. Paired DNA samples were extracted from the

blood and tumor of the patients and analyzed for chromosome 22 LOH. The presence of mutations was screened for in all 17 exons of the NF2 gene by SSCP, and PCR products showing aberrant SSCP bands were subsequently sequenced by conventional DNA sequencing method.

Fourteen (61%) of 23 meningiomas showed chromosome 22 LOH and 8 meningiomas (35%) showed somatic NF2 gene mutations. No abnormality was detected in hemangioblastoma and hemangiopericytoma. In vestibular schwannoma study, chromosome 22 LOH was observed in 35 (40%) of 87 cases available for the study, and NF2 gene mutational analysis revealed 40 mutations in 36 tumors (38%). The total number of the cases in which either chromosome 22 LOH or NF2 gene mutation was identified amounted to 52 (55%).

The results of the study have confirmed the hypothesis that both meningioma and vestibular schwannoma share a common molecular genetic basis of tumorigenesis. In both tumors, the NF2 gene acts as a tumor suppressor gene and the mechanism of the tumorigenesis is consistent with the "two hit" mutation model of Knudson. The inactivation of the NF2 gene is important in the pathogenesis of not only NF2-related tumors but also in sporadic meningioma and vestibular schwannoma.

(Note: The molecular genetic investigations described in this paper were conducted at the Molecular Genetics Laboratory of Addenbrooke's Hospital in Cambridge, U.K. The author expresses his deep appreciation particularly to Mr. Richard M Irving and also to all the members of the Lab for their kind helps.)

References

1) Russell DS, Rubinstein LJ (eds): Pathology of Tumours of the Nervous System. 5th ed. Edward Arnold, London, 1989.
2) Moffat DA, Saunders JE, McElveen JT, et al: Unusual cerebello-pontine angle tumours. J Laryngol Otol 107:1087-1098, 1993.
3) Evans DGR, Huson SM, Donnai D, et al: A genetic study of type 2 neurofibromatosis in the United Kingdom. II. Guidelines for genetic counselling. J Med Genet 29:847-852, 1992.
4) Sutherland GR, Florell R, Louw D, et al: Epidemiology of primary intracranial neoplasms in Manitoba, Canada. Can J Neurol Sci 14:586-592, 1987.
5) Tos M, Thomsen J: Epidemiology of acoustic neuromas. J Laryngol Otol 98:685-692, 1984.
6) von Recklinghausen F: Ueber die multiplen Fibroma der Haut und ihre Beziehung zu den multiplen Neuromen. A Hirschwald, Berlin, 1882.

7) Seizinger BR, Rouleau GA, Ozelius LG, et al: Genetic linkage of von Recklinghausen neurofibromatosis to the nerve growth factor receptor gene. Cell 49:589-594, 1987.
8) Rouleau GA, Wertelecki W, Haines JL, et al: Genetic linkage analysis of bilateral acoustic neurofibromatosis to a DNA marker on chromosome 22. Nature 329:246-248, 1987.
9) Evans DGR, Huson SM, Donnai D, et al: A genetic study of type 2 neurofibromatosis in the United Kingdom. I. Prevalence, mutation rate, fitness, and confirmation of maternal transmission effect on severity. J Med Genet 29:841-846, 1992.
10) Rouleau GA, Mérel P, Lutchman M, et al: Alteration in a new gene encoding a putative membrane-organizing protein causes neurofibromatosis type 2. Nature 363:515-521, 1993.
11) Trofatter JA, MacCollin MM, Rutter JL, et al: A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell 72:791-800, 1993.
12) Bianchi AB, Hara T, Ramesh V, et al: Mutations in transcript isoforms of the neurofibromatosis 2 gene in multiple human tumour types. Nature Genet 6:185-192, 1994.
13) Knudson AG Jr: Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci USA 68:820-823, 1971.
14) Lekanne Deprez RH, Groen NA, van Biezen NA, et al: A t(4;22) in a meningioma points to the localization of a putative tumor-suppressor gene. Am J Hum Genet 48:783-790, 1991.
15) Seizinger BR, Martuza RL, Gusella JF: Loss of genes on chromosome 22 in tumorigenesis of human acoustic neuroma. Nature 322:644-647, 1986.
16) Ruttledge MH, Sarrazin J, Rangaratnam S, et al: Evidence for the complete inactivation of the NF2 gene in the majority of sporadic meningiomas. Nature Genet 6:180-184, 1994.
17) Ng HK, Lau KM, Tse JY, et al: Combined molecular genetic studies of chromosome 22q and the neurofibromatosis type 2 gene in central nervous system tumors. Neurosurg 37:764-773, 1995.
18) Wellenreuther R, Kraus JA, Lenartz D, et al: Analysis of the neurofibromatosis 2 gene reveals molecular variants of meningioma. Am J Path 146:827-832, 1995.
19) de Vitis LR, Tedde A, Vitelli F, et al: Screening for mutations in the neurofibromatosis type 2 (NF2) gene in sporadic meningiomas. Hum Genet 97:632-637, 1996.
20) Lee JH, Sundaram V, Stein DJ, et al: Reduced expression of schwannomin/merlin in human sporadic meningiomas. Neurosurg 40:578-587, 1997.

21) Ueki K, Wen-Bin C, Narita Y, et al: Tight association of loss of merlin expression with loss of heterozygosity at chromosome 22q in sporadic meningiomas. Cancer Res 59:5995-5998, 1999.
22) Carlson KM, Bruder C, Nordenskjold M, et al: 1p and 3p deletions in meningiomas without detectable aberrations of chromosome 22 identified by comparative genomic hybridization.
Genes Chromosomes Cancer 20:419-424, 1997.
23) Kimura Y, Koga H, Araki N, et al: The involvement of calpain-dependent proteolysis of the tumor suppressor NF2 (merlin) in schwannomas and meningiomas. Nat Med 4:915-922, 1998.
24) Leone PE, Bello MJ, de Campos JM, et al: NF2 gene mutations and allelic status of 1p, 14q and 22q in sporadic meningiomas.
Oncogene 18:2231-2239, 1999.
25) Jacoby LB, MacCollin M, Louis DN, et al: Exon scanning for mutation of the NF2 gene in schwannomas. Hum Mol Genet 3:413-419, 1994.
26) Sainz J, Huynh DP, Figueroa K, et al: Mutations of the neurofibromatosis type 2 gene and lack of the gene product in vestibular schwannomas.
Hum Mol Genet 3:885-891, 1994.
27) Mérel P, Hoang-Xuan K, Sanson M, et al: Predominant occurrence of somatic mutations of the NF2 gene in meningiomas and schwannomas.
Genes Chromosomes Cancer 13:211-216, 1995.
28) Irving RM, Harada T, Moffat DA, et al: Somatic neurofibromatosis type 2 gene mutations and growth characteristics in vestibular schwannoma.
Am J Otol 18:754-760, 1997.